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Research Note

Use of Vacuum-Steam-Vacuum and Ionizing Radiation To Eliminate *Listeria innocua* from Ham

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ABSTRACT

Listeria spp. are a frequent postprocess contaminant of ready-to-eat (RTE) meat products, including ham. Vacuum-steam-vacuum (VSV) technology has been used successfully to eliminate *Listeria innocua* from hot dogs. Ionizing radiation can eliminate *Listeria* spp. from RTE meats. However, the excessive application of either technology can cause changes in product quality, including structural changes, changes in cure color (redness), and lipid oxidation. In this study, two cycles of VSV were combined with 2.0 kGy of ionizing radiation to obtain 4.40- and 4.85-log₁₀ reductions of *L. innocua* on ham meat and skin, respectively. The use of both treatments resulted in an additive, as opposed to synergistic, reduction of *L. innocua* on ham. The combination treatment did not cause statistically significant changes in product structure, color (redness), or lipid oxidation.

Listeria spp. are a frequent postprocess contaminant of ready-to-eat (RTE) meat products (8). *Listeria monocytogenes*, a pathogen, has been associated with a number of foodborne illness outbreaks and food product recalls (1, 2) and is given zero tolerance in RTE meat products because of the high mortality rate associated with listeriosis (6, 11). Ionizing radiation can eliminate *Listeria* spp. from RTE meat products (9, 10). However, ionizing radiation can sometimes reduce the redness of RTE meats or produce small increases in lipid oxidation (9). Kozempel et al. (4) demonstrated the use of the vacuum-steam-vacuum (VSV) system for the elimination of *L. innocua* from hot dogs. Unfortunately, this system can sometimes result in physical damage to the product if too many VSV cycles are used (4, 5).

In this study, we investigated the ability of VSV in combination with ionizing radiation to eliminate the *L. monocytogenes* surrogate *Listeria innocua* from ham meat and ham skin. *L. innocua* was used because the VSV prototype apparatus is located in an open-air pilot plant, not in a microbiology laboratory. The following questions were addressed: (1) How many cycles of VSV can ham tolerate, and how large a reduction of *L. innocua* is obtained with VSV? (2) How large a reduction of *L. innocua* would be obtained with the application of sublethal doses of ionizing radiation following VSV and vacuum packaging? (3) What are the effects of these two processes on product color and lipid oxidation?

MATERIALS AND METHODS

Microbiology. Three *L. innocua* strains (33090, 33091, and 52871) were obtained from the American Type Culture Collection.

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The protocol of Sommers and Thayer (9, 10) was used to propagate and concentrate *L. innocua* strains. The D_{10} -value (90% reduction dose) of the *L. innocua* strains surface inoculated onto ham was determined to be 0.56 kGy, or well within the 0.49-to-0.71-kGy range established by Sommers and Thayer (9, 10) for *L. monocytogenes*. Commercially prepared ham sections (with surface areas of 225 cm² per side) that contained skin on at least one face were surface inoculated by pipetting and spreading 8 log₁₀ CFU of *L. innocua* suspension with a sterile swab, and these ham sections were then placed in No. 400 Stomacher Bags (Tekmar, Inc., Cincinnati, Ohio). The ham was maintained in a refrigerated cooler (for 30 to 60 min) prior to VSV or irradiation.

After VSV or irradiation treatment the ham pieces were diluted in 100 ml of Butterfield's phosphate buffer (Applied Biosciences, Newtown, Conn.) and mixed by shaking for approximately for 90 s. The diluents were then serially diluted in Butterfield's phosphate buffer with 10-fold dilutions, and 1-ml aliquots of the appropriately diluted samples were pour plated onto Palcam agar. Three 1-ml aliquots were plated for each dilution. The plates were then incubated for 48 h at 37°C prior to scoring. Log₁₀ reduction values for *L. innocua* were determined as previously described (9, 10).

VSV surface intervention processor mechanical design.

The mechanical design and operation of the VSV processor have previously been described by Kozempel et al. (4). The surface intervention processor was originally designed to process chicken carcasses (specifically, broilers) but can be used for other solid food products. The processor accepts product samples individually and encloses them in a chamber within a rotor, evacuates the chamber, injects saturated steam into the chamber, draws vacuum on the chamber to evaporatively cool the product, and, finally, ejects the sample into a clean environment. A prototype field unit containing one chamber in one rotor was designed and constructed. To accommodate the deli ham samples, a cylindrical wire basket was installed in the product treatment chamber. The basket

was 152 mm in diameter and 152 mm deep and was enclosed with screening containing 12-mm openings. The spherical chamber (product valve) is 254 mm in diameter.

To admit vacuum or steam into the closed chamber, two opposing 200-mm holes were bored through the ball valve stator (housing) at right angles to both the axis of rotation of the ball and the centerline of the open chamber (the product entry and exit ports). Two platter valves are close coupled to these 200-mm ports; each consists of a flat disk with two holes or ports rotating against an inlet header that holds polyetheretherketone seals. When the disk is aligned with the ports in the inlet header, gas flows into or out of the treatment chamber. Multiple holes reduce the disk angular movement necessary for valve action and increase the cross-sectional area for gas flow.

Each disk is programmed and controlled independently and moved by its own servomotor. The servos (Allen-Bradley Co., Inc., Mayfield Valley, Ohio) are capable of rapid acceleration and deceleration. The servos for the disks (model 1326AB-C4B-11; 5.6 kW) are directly coupled mechanically to the disks and are capable of a maximum speed of 1,600 rpm. The vacuum and steam times are controlled with the servos, which are controlled by Graphics Motion Language software (version 3.8.2, Allen-Bradley). The Lab Tech Notebook (version 8.04, Laboratory Technologies Corp., Wilmington, Mass.) was used for data acquisition. Sensors were made by Omega Engineering, Inc. (Stamford, Conn.). Type E thermocouples were used for temperature measurement, and Omega PX176 series sensors were used for vacuum and steam pressure.

Vacuum was supplied by a liquid ring vacuum pump (Model HER, Stokes Vacuum, Philadelphia, Pa.). The steam generator was a 115-liter tank consisting of horizontal submerged coils with no separator and 17.8-kW heaters; it was fabricated in-house and charged with water that was boiled for 30 min for deaeration. The vacuum receiver was adjusted to 7 kPa, and its condenser coil was cooled to 4°C.

VSV surface intervention processor operation. Each ham sample was removed from the stomacher bags and manually inserted into the treatment chamber of the surface intervention processor. The computer-controlled ball valve was rotated 90° with a servo to seal the chamber from the outside atmosphere. The platter valves in the main chamber rotated to expose the sample to vacuum, then to steam, and then to vacuum again. With multiple cycles, the VSV sequence was repeated multiple times. After treatment, the ball valve rotated 90° to expose the sample to the atmosphere. The sample was aseptically removed manually after treatment and placed in a new stomacher bag. The ham samples were vacuum packed to 133.322 Pa with a Multi-Vac Model A300 packager (Multi-Vac, Kansas City, Mo.) and stored at 0 to 2°C until irradiation. Irradiation was begun within 1 h of inoculation for all experiments.

Irradiation. A temperature-controlled (4°C) self-contained ^{137}Cs gamma irradiation source (Lockheed Georgia Co., Marietta, Ga.) was used for all experiments (9, 10). The dose rate provided by the irradiator was 0.096 kGy/min. The absorbed radiation doses were verified with alanine dosimeters and were then measured with a Bruker 104 EPR Analyzer (Bruker, Inc., Billerica, Mass.) (9, 10).

Lipid oxidation and color measurement. Ham pieces were vacuum packaged and processed as described above. Lipid oxidation of ham meat and ham skin samples (3 by 10 g) was measured with the thiobarbituric acid-reactive substances assay modified from Hodges et al. (3) and Zipser and Watts (12). Color

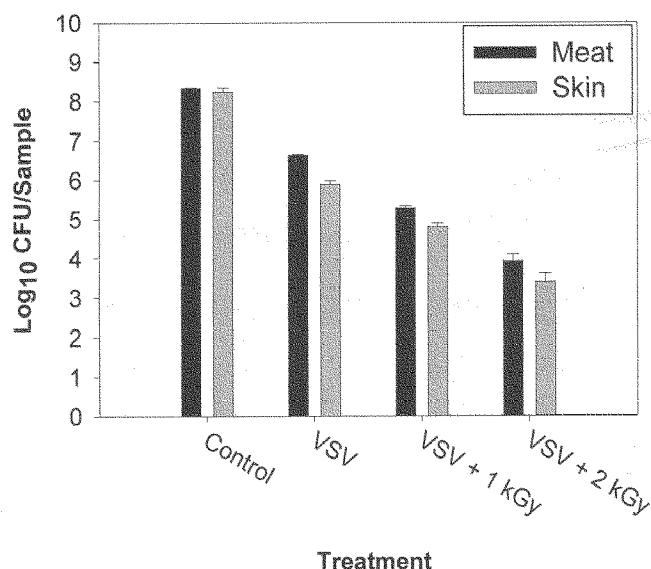


FIGURE 1. \log_{10} reductions of a three-strain mixture of *L. innocua* surface inoculated onto ham meat or ham skin following exposure to VSV and either 1.0 or 2.0 kGy of ionizing radiation. Standard error bars are shown for each parameter tested. Each experiment was conducted independently three times.

analysis was carried out with a Hunter Miniscan XE meter (Hunter Laboratory, Inc., Reston, Va.) as outlined by Nanke et al. (7).

RESULTS AND DISCUSSION

Prior to the microbial analysis, ham sections were subjected to either two or three cycles of VSV (with 138°C steam) to determine the effect of the process on product integrity. Three cycles of VSV caused occasional separation of the ham skin from the meat or separation of the ham meat along connective tissue lines. Two VSV cycles (138°C steam for 0.1 s, vacuum for 0.1 s, 138°C steam for 0.1 s, vacuum for 0.5 s) did not cause damage to the 18 ham samples used in the microbial analysis.

The ability of two cycles of VSV to eliminate *L. innocua* that was surface-inoculated onto either ham meat or ham skin is illustrated in Figure 1. Two cycles of VSV resulted in a 1.69- \log_{10} reduction of *L. innocua* on ham meat and a 2.35- \log_{10} reduction of the pathogen on ham skin. These reductions were significantly lower than the 5- \log_{10} reduction obtained for *L. innocua* surface inoculated onto hot dogs in a previous study (4). *L. innocua* inoculated onto ham skin was eliminated to a greater degree than was *L. innocua* inoculated onto ham meat as determined by analysis of variance ($n = 3$, $\alpha = 0.05$). With two cycles of VSV and 2.0 kGy of ionizing radiation, a 4.40- \log_{10} reduction of *L. innocua* on ham meat and a 4.85- \log_{10} reduction of *L. innocua* on ham skin were obtained (Fig. 1). The 2.5- to 3.0- \log_{10} reduction of *L. innocua* obtained with 2.0 kGy of ionizing radiation is consistent with data obtained by Sommers and Thayer (10) for *L. monocytogenes*. The radiation D_{10} -value for the *L. innocua* mixture inoculated onto ham meat and ham skin, 0.56 kGy, was well within the 0.49-to-0.71-kGy range of D_{10} -values obtained for *L. monocytogenes* inoculated onto RTE meats (10). The ability of VSV and irradiation together to eliminate *L. innocua* from ham was additive, as opposed to synergistic.

TABLE 1. Color analysis and lipid oxidation measurements for ham treated with VSV and ionizing radiation^a

Treatment	L	a	b	TBARS value
Meat				
Control	60.2 ± 3.39	11.0 ± 0.90	9.55 ± 0.89	6.49 ± 0.41
VSV (2 cycles)	60.9 ± 3.91	10.5 ± 1.53	9.62 ± 0.87	6.48 ± 0.49
Radiation (2.0 kGy)	57.5 ± 9.86	11.9 ± 4.16	13.2 ± 7.31	6.60 ± 0.31
VSV + radiation	56.8 ± 3.89	11.6 ± 1.42	10.1 ± 0.38	6.66 ± 0.27
Skin				
Control	38.9 ± 3.39	18.1 ± 1.87	25.2 ± 3.09	5.75 ± 0.58
VSV (2 cycles)	44.5 ± 4.96	18.8 ± 1.65	33.2 ± 6.20	5.41 ± 0.24
Radiation (2.0 kGy)	39.5 ± 3.18	19.9 ± 1.74	30.1 ± 2.38	5.21 ± 0.21
VSV + radiation	45.1 ± 3.28	16.1 ± 1.60	28.5 ± 4.94	5.46 ± 0.25

^a Hunter color analysis results (L, brightness value; a, redness value; b, yellowness value) are presented as means ± standard errors of the mean for six individual readings. Lipid oxidation measurements (μg of malondialdehyde per g), as determined with the thiobarbituric acid–reactive substances [TBARS] assay), are presented as means ± standard errors of the mean of three values.

To determine the effect of VSV and ionizing radiation on product appearance, a color analysis was carried out (Table 1). No statistically significant difference was observed in the ham meat's cure color as a result of either treatment for the L value (brightness), the a value (redness), or the b value (yellowness) as determined by analysis of variance ($n = 6$, $\alpha = 0.05$). The L value for the ham skin increased by approximately 1 unit as a result of VSV treatment as determined by analysis of variance ($n = 6$, $\alpha = 0.05$); however, the increase was not visually perceptible to the researchers. This finding is consistent with Sommers et al.'s (9) finding that the redness of RTE meats was reduced by 4 kGy of ionizing radiation but not by 2.0 kGy. No statistically significant differences in lipid oxidation were observed for the parameters examined (Table 1). This finding is consistent with previous findings that 3.5 kGy of ionizing radiation induced lipid oxidation in RTE meats, but 1.75 kGy did not (9).

The application of three VSV cycles was determined to be inappropriate for the treatment of the ham pieces. Treatment with two cycles of VSV produced visually and structurally acceptable ham but was not effective in eliminating *L. innocua* from the product surface. The application of two cycles of VSV followed by 2 kGy of ionizing radiation resulted in a 4- to 5-log₁₀ reduction of *L. innocua* without negatively affecting ham quality. Results indicate that the combination of VSV and ionizing radiation treatment would be useful for circumstances under which one process alone could cause undesirable effects on product quality.

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